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INSTITUT FÜR PFLANZENGENETIK UND
KULTURPFLANZENFORSCHUNG,
Corrensstrasse 3
D-06466, GATERSLEBEN, XX (DE).

(72)

HEIM, UTE (DE).
WEBER, HANS (DE).

(74)

DEETH WILLIAMS WALL LLP

- (54) NOUVELLE CASSETTE D'EXPRESSION PERMETTANT D'EXPRIMER DES GENES QUELCONQUES DE
GRAINES DE PLANTES
- (54) NOVEL EXPRESSION CASSETTE FOR EXPRESSING GENES IN PLANT SEED

(57)

The invention relates to an expression cassette for expressing genes in plant seed and to the plasmids containing said expression cassette. The invention includes the production of transgenic plant cells containing said expression cassette and the use of the plasmids in said expression cassette for producing transgenic plants. The invention can be applied in the field of biotechnology, pharmaceuticals and plant production. The aim of the invention is to provide a means for the seed-specific expression in transgenic plants in such a manner that it is suitable for the production of the desired substances. Another aim of the invention is to construct an expression cassette which allows stable expression of genes of substances to be produced in plant seed at a high expression rate. The inventive expression cassette comprises the following essential components: the promoter of the gene of the seed protein which is analogous to the sucrose binding protein (SBP), optionally the DNA sequence of a signal peptide, preferably that of the SBP signal peptide, a gene to be expressed, 3' termination sequences.



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(71) Demandeur/Applicant:
INSTITUT FÜR PFLANZENGENETIK UND
KULTURPFLANZENFORSCHUNG, DE

(72) Inventeurs/Inventors:
WEBER, HANS, DE;
HEIM, UTE, DE

(74) Agent: DEETH WILLIAMS WALL LLP

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(54) Title: NOVEL EXPRESSION CASSETTE FOR EXPRESSING GENES IN PLANT SEED

(57) Abrégé/Abstract:

The invention relates to an expression cassette for expressing genes in plant seed and to the plasmids containing said expression cassette. The invention includes the production of transgenic plant cells containing said expression cassette and the use of the plasmids in said expression cassette for producing transgenic plants. The invention can be applied in the field of biotechnology, pharmaceuticals and plant production. The aim of the invention is to provide a means for the seed-specific expression in transgenic plants in such a manner that it is suitable for the production of the desired substances. Another aim of the invention is to construct an expression cassette which allows stable expression of genes of substances to be produced in plant seed at a high expression rate. The inventive expression cassette comprises the following essential components: the promoter of the gene of the seed protein which is analogous to the sucrose binding protein (SBP), optionally the DNA sequence of a signal peptide, preferably that of the SBP signal peptide, a gene to be expressed, 3' termination sequences.

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Abstract

The present invention relates to an expression cassette for expression of arbitrary genes in plant seed and the plasmids containing the expression cassette. The invention also includes the production of transgenic plant cells containing this expression cassette as well as the use of the plasmids in this expression cassette to produce transgenic plants. Fields of application of the invention are biotechnology, pharmacy and plant production.

The invention has the objective of placing seed-specific expression in transgenic plants on a basis suitable for a production of materials. It is based on the task of constructing an expression cassette with which a stable expression with a high expression rate of genes of the materials to be produced can be achieved in plant seeds.

The expression cassette according to the invention contains the following essential component parts:

- the promoter of the gene of the seed protein similar to the sucrose binding protein (SBP)
- if applicable, the DNA sequence of a signal peptide, preferably the SBP signal peptide
- a gene to be expressed
- 3' termination sequences

New expression cassette for expression of arbitrary genes in plant seeds

Description

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The invention in question relates to an expression cassette for expression of arbitrary genes in plant seeds and the plasmids containing the expression cassette. The invention also includes the production of transgenic plant cells containing this expression cassette as well as the use of the plasmids in this expression cassette for production of transgenic plants. Fields of application of the invention are biotechnology, pharmacy and plant production.

15 For a long time now, there have been methods making it possible to integrate relevant genes into the genome of higher plants. The objective of this work is the production of plants with new properties, for example to increase agricultural production, to optimise manufacture of foodstuffs and to produce specific pharmaceuticals and other interesting ingredients. One prerequisite for the expression of the transferred genes in this context is that they possess plant-specific promoter sequences. For this purpose, so-called constitutive promoters such as the promoter of the nopaline synthase gene /1/, the TR double promoter /2/ or the promoter of the 35S transcript of the cauliflower mosaic virus /3/ are used. One disadvantage of these promoters is that they are active in almost all the tissues of the manipulated plants. In this way, a controlled and purposeful expression of the foreign genes in the plants is not possible. It is better to use promoters which function tissue-specifically and independently of development. Genes with the matching promoters, which are only active in anthera, ovaries, blooms, leaves, deciduous leaves, stems, roots or seeds, have been isolated /4/. But they differ greatly in the strength and specificity of the expression and only have a limited use. For the use of the seeds as a source of nutrition and for production of in-

gredients, it is above all the seed-specific promoters which are of great interest. With the years of research into the genes of the seed-storage proteins, some more or less specific promoters with differing strengths, for example that of phaseolin /5/ or legumin and USP /6/ are available. As these storage proteins are synthesised by gene families, fusions of such promoters with foreign genes are in competition with the endogenous numerous genes of the corresponding gene family. For this reason, it is more favourable to use promoters from unique, strongly and specifically expressing genes. For co- and multiple transformations, the use of differing regulatory sequences is suitable, in order to make better use of the development of the seed in time, to synthesise identical or differing gene products in parallel and to avoid co-suppression.

Although a number of expression cassettes for expression of arbitrary genes in plant seeds are already known, the expression rates in plant seeds achieved have not been optimal up to now for the substantiation of a plant biotechnological production of the required materials.

The invention therefore has the objective of placing the seed-specific expression in transgenic plants on a basis suitable for a production of materials. It is based on the task of constructing an expression cassette with which a stable expression with a high expression rate of genes of the materials to be produced can be achieved in plant seeds.

The objective of the invention is achieved with the expression cassette described in claim 1, with sub-claims 2-7 being preferred variants.

The expression cassette according to the invention contains the following essential component parts:

- the promoter of the gene of the sucrose binding protein (SBP)like protein

- if applicable, the DNA sequence of a signal peptide, preferably the SBP signal peptide
 - a gene to be expressed
 - 3' termination sequences
- 5 The invention relates above all to a regulatory DNA sequence occurring uniquely in the genome, which mediates a strong expression of an arbitrary heterologous gene primarily in the cotyledons and in the endosperm dependency on seed development.
- 10 The most important component part of the cassette is the SBP promoter, the sequence of which is shown in Figure 1. Compared with analog promoters in this field, this promoter has the benefit of great strength and seed-specificity. Its use for the expression of foreign genes, even without the DNA sequence of a signal peptide, is also part of the scope of the
- 15 invention.

Together with the transcriptionally regulatory sequences, the expression cassette also, if need be, contains a signal peptide, which enables the transport of the required gene product into the protein bodies, thus preventing decomposition of the gene products to a great extent. The optional use of the authentic signal peptide enables the transport of the synthesised foreign proteins to and storage in the protein bodies.

25 The genes to be expressed can be integrated either as transcription or as translation fusions, they can be varied to a great extent, for example genes can be used for the production of enzymes (e.g. amylase, xylanase), pharmaceutical

30 products or for the over-expression of proteins with a high share of essential amino-acids (e.g. 2S globulin of the brazil nut rich in methionine) or of other proteins influencing the properties of the seeds. Further possibilities can be found in the reduction or elimination of gene products

35 through the integration of genes in an anti-sense orientation. By inserting regulatory genes under the control of this seed-specific promoter, metabolic processes in the seeds can

also be influenced. The cassette can also be used in order to express the SBP gene inherent to the promoter from field beans into other species. The use of other terminators, for example the termination sequence of the gene to be expressed, is a further possibility of optimal use of the cassette. As a concrete example, the gene of β -glucuronidase (GUS) was used to show the specificity of the promoter (Fig. 2b, c).

The nucleotide sequence of the expression cassette contains transcriptionally regulatory areas, guaranteeing a strong specific expression of an arbitrary gene into the seed of plants. The Northern (Fig. 2a) shows the high seed-specific expression in the various tissues of *Vicia faba*. The GUS data in Figs. 2b and 2c show on the one hand the distribution of the β -glucuronidase in the sections through ripe tobacco seeds and, on the other, the accumulation of the β -glucuronidase in the transgenic tobacco seeds as a function of development.

The plasmids containing the expression cassette, preferably the plasmids pSBPROCS and pPTVSBPRGUS, are also to be placed under protection.

The scope of the invention also includes the use of the expression cassette according to claims 12-16, which is done by transformation into bacteria strains and subsequent transfer of the resulting recombinant clones into preferably dicotyl plants. The plants expressing the required gene product in the seed are selected and bred as genetically stable lines. After harvesting, the required gene products are extracted from the transgenic seeds in a way basically already known.

This invention is also interesting for applications in which the required gene product is expressed under the control of various promoters, in order to increase the total of the expression rates, in order to make better use of the development period of the seeds and to avoid effects by co-suppression. This expression cassette is also suited for co- and

multiple transformations with the objective of expressing various gene products. A variety of new expression cassettes is needed for these strategies in order to be able to select the correct ones.

5 The entire method for the alteration of a plant cell is portrayed in an example (pSBPOCS).

10 The invention is to be explained in more detail below with examples of embodiments.

Methods

1. Cloning method

15 For cloning, the vectors pUC18 /7/, pBK-CMV (Stratagene) and pOCS1 (Plant Genetic Systems, Gent, Belgium) and for plant transformation the vectors BIN19 /8/, and, after deletion of the GUS gene, pGPTV-BAR /9/ were used.

20 2. Bacteria strains

For the transformation to E. coli, strain DH5 α /10/ was used. The binary plasmids were inserted into the agro-bacteria strain EHA105 /11/ by conjugation.

25 3. Plant transformation

The transformation of *Nicotiana tabacum* was done by the leafdisk method /12/ and the transformation of *Vicia narbonensis* with the help of the method described by Pickardt in 1991 /13/ by agrobacterium mediated gene transfer.

30

4. Analysis of genomic DNA from transgenic plants

The genomic DNA of the transgenic tobacco and *V. narbonensis* plants was isolated with the help of the DNA isolation kit of the firm of Macherey & Nagel. In a first step, the transgenic
35 lines were identified via PCR with gene-specific primers. The integration of foreign DNA was examined by means of "Southern

blot" analyses of 20 μ g of DNA following suitable restriction digestion.

5. β -glucuronidase activity test (GUS assay)
- 5 The reporter gene β -glucuronidase is a bacterial enzyme accessible to both quantitative /14/ and also histo-chemical activity assays. Tissue samples were incubated over night at 37°C in 1 mM X-Gluc, 50mM Na phosphate (pH 7.0) and 0.1% Tween 20. For sections, the tissues were fixed, embedded in
- 10 paraffin and cut to a section thickness of 15 - 30 μ m on a microtome.

Examples of embodiments

- 15 The invention, which contains the production of a new, seed-specific expression cassette as well as the plasmids and transgenic plants derived from them, is explained below - partly with the help of the figures - using an example of an embodiment.
- 20 1.) Cloning and structure analysis of an SBP seed protein gene from *Vicia faba*
- Primers (5'-GAAGACCCTGAGCTCGTAACTTGCAA-ACAC- 3' and 5'-AGTACTCATAGATCTCTGGGTGATGTTGGT-3') were derived from the sequence of a cDNA clone which codes for the sucrose binding
- 25 protein of the soybean /15/. The gene-specific probe was then amplified, cloned and sequenced by means of RT - PCR on mRNA, isolated from immature cotyledons of *V. faba*. The PCR product was identified as the gene fragment homologous to the sucrose
- 30 binding protein and was used as a probe for the isolation of the complete cDNA from a cotyledon-specific λ Zap Express cDNA Bank of *V. faba* L. var. minor. One of the isolated clones (VfSBP20), which has a homology of 68% on the nucleotide level, codes for the complete SBP-homologous gene
- 35 from the field bean. But it differs from the gene isolated from the soybean in both the expression (Fig. 2a) and also in the function (no sucrose binding).

2) Isolation of the regulatory sequences by means of PCR
 The regulatory sequences were isolated with the help of the
 "Universal GenomeWalker™Kit" of the firm of CLONTECH and the
 5 gene-specific primers PSBP1, position 159 (5'-AATCCTCA-
 CACTTCTCCATGCATATCCGTTTGTCC-3'), PSBP2, position 118 (5'-
 GCCCTGCAGAT-CGCATTTGTCTTTGCA-3') and PSBP3, position 85 (5'-
 CTGGGTCCTTTTCTTTTCTGG- C-3'). Following prior digestion of
 the genomic DNA of *V.faba* with *ScaI* (a) and *StuI* (b) and
 10 ligation of the adapters, a two-step PCR was done in accor-
 dance with the description of the kit with the following pa-
 rameters: 7 cycles of 94°C, 2s, 72°C, 3 min and 32 cycles of
 94°C, 2s, 67°C, 3 min and 4 min 67°C. The PCR preparations
 were diluted 1:50 and 1µl of each were amplified in a second
 15 PCR (5 cycles of 94°C, 2s, 72°C, 3 min and 20 cycles of 94°C,
 2s, 67°C and 4 min at 67°C. In the Agarosegel, bands of 1.7 kb
 from (a) and 1.9 kb from (b) were verified via a Southern
 blot. These bands were then cloned into the pUC18 and se-
 quenced. The clones SBPR7 and SBPR15 were then identified by
 20 a sequence comparison as the promoters matching gene *VfSBP20*.
 They represent allelic variants of gene *VfSBP20*, with both
 clones having 100% sequence identity with clone *VfSBP20* in
 the corresponding area. On the 5' side of the ATG of the SBP
 gene, 1539 bp were isolated with clone SBPR7 and 1750 bp with
 25 clone SBPR15. They differ by 23 base pair substitutions and
 two insertions. The restriction maps of clone pSBPR7 and
 pSBPR15 are shown in Fig. 3, the sequence of clone pSBPR15 in
 Fig. 1.

30 3a) Proof of the seed-specific expression in tobacco
 With the help of the reporter gene of β -glucuronidase, the
 seed-specific expression of the isolated regulatory sequences
 SBPR7 and SBPR15 was to be tested. For this, the binary plas-
 mid pBI101 /14/, which contains the promoter-free glucuroni-
 35 dase gene behind a poly-linker, was cut with *SmaI* and dephos-
 phorylated. The promoters were isolated from the plasmids
 pSBPR7 and pSBPR15 respectively by means of an *SalI*/*NcoI*

digestion and the ends smoothed. The fragments were then cloned into the SmaI site of binary plasmids pBI101 in front of the reporter gene, with plasmids pBISBPR7GUS and pBISBPR15GUS resulting. These plasmids were then transferred to the agro-bacteria strain EHA105 and the chimerical agro-bacteria containing SBP promoter/glucuronidase gene were used for the transformation of tobacco. The results are shown in Figures 2b and 2c. The analysis of the transgenic tobacco seeds shows a strong blue coloration and thus a strong activity of the glucuronidase in the endosperm and in the cotyledons of the tobacco seeds, also according to the seed development. No glucuronidase activity was detected in other tissues. The two slightly different nucleotide sequences SBPR7 and SBPR15 also do not differ in their expression behaviour. These data show that the isolated regulatory sequences fused with the β -glucuronidase gene result in a strong and strictly seed-specific expression in the tobacco.

3b) Proof of the seed-specific expression in peas
 In order to show that a seed-specific expression is also to be expected in legumes, the SalI/NcoI fragment of plasmid pSBPR15 was cloned into the SalI/NcoI cut plasmid pGUS1 (Plant Genetic Systems, Gent). From the resulting plasmid pSBPGUS, the fusion of the SBPR15 promoter/GUS/ocs-terminator was cut out with SalI/SmaI, smoothed and ligated into the binary plasmid pGPTV-Bar, EcoRI/SmaI cut (Fig. 4). pGPTV-Bar /9/ is a binary plasmid mediating phosphinothricin resistance which is successfully used for the transformation of peas. This plasmid has been called pPTVSBPRGUS (Fig. 4). The embryos of the transgenic pea lines generated with this plasmid show a strong blue coloration after a histo-chemical analysis.

3c) Proof of the transient expression in embryos of *Vicia faba*, *Vicia narbonensis*, *Pisum sativum* and *Brassica napus*
 With plasmid pSBPGUS, isolated embryos of *Vicia faba*, *Vicia narbonensis*, *Pisum sativum* and *Brassica napus* were shot by

means of the Biolistics PDS-1000/He Particle Delivery System under the following conditions. The coating preparation comprised 50 μ l of gold (Hereaus, 0.6-3 μ m, 50 mg/ml), 10 μ l of Qiagen-cleaned plasmid-DNA (1 μ g/ μ l), 50 μ l of 2.5M CaCl₂ and 10 μ l of 0.1M spermidine. At 1800 Psi and a vacuum of 27 inch Hg, the embryos lying on an agar panel were then shot and subsequently cultivated in MS-2% sucrose liquid medium for 2 days. There was then a reaction over night at 37°C with X-Gluc (1mM) in 50mM Na phosphate (pH 7.0) and 0.1% Tween 20. Unlike the negative control (promoter-free pGUS1), a number of blue dots were registered in the above mentioned embryos, showing that the SBP promoter functions in the seeds.

4.) Production of the expression cassette for over-expression of heterologous genes in the seed

In order to make the regulatory sequences available for the over-expression of foreign genes, the SalI fragment of the longer clone SBPR15 was isolated and smoothed and cloned into the SmaI location of plasmid pOCS1 (Plant Genetic Systems, Gent, Belgium). This cassette thus contains the promoter region, the complete 5' untranslated region, the complete signal peptide, the first five triplets of the ripe protein (Fig. 1) and the 3' untranslated area with the polyadenylation signals of the octopine synthase gene (Fig. 5). The NcoI location can be used for transcription fusions with foreign genes, the BamHI location for translation fusions. After the insertion of the foreign gene, the sequence containing the promoter, regulatory sequences, the foreign gene and the 3' termination sequences is cut out with restriction enzymes and cloned into a binary vector with the herbicide resistance suitable for the plant transformation.

As an example of this, the BamHI fragment of the gene of XylanaseZ of Clostridium thermocellum was cloned into the BamHI location of plasmid pSBPOCS as a translation fusion. From the resulting plasmid pSBPRXNZ (Fig. 6), the smoothed Asp718/SphI fragment was ligated with the binary vector

pgPTV-Bar, which was cut with the enzymes EcoRI/SmaI and smoothed. After transformation into the agro-bacteria strain EHA105, *N. Tabacum* was transformed. The strong expression of the Xylanase Z was shown in the ripe transgenic seeds in a Western blot (Fig. 7).

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Patent claims

1. Promoter for expression of arbitrary genes in plant seeds,
wherein there exists the sequence of Fig. 1a, which thus
becomes the object of the claim.
2. Promoter according to claim 1, wherein it mediates the ex-
pression in the cotyledons and in the endosperm of seeds as
a function of development.
3. Expression cassette for expression of arbitrary genes in
the plant seed, containing:
 - a promoter according to claim 1 or 2,
 - a gene to be expressed
 - 3' termination sequences.
4. Expression cassette according to claim 3, wherein it addi-
tionally contains the DNA sequence of a signal peptide,
preferably the SBP signal peptide.
5. Expression cassette according to claim 3, wherein a further
DNA sequence is downstream to the DNA region provided with
a transcriptionally regulatory sequence for a strong seed-
specific gene expression, the latter region containing the
information for the formation and quantitative distribution
of endogenous products or the expression of heterologous
products in culture crops.
6. Expression cassette according to claims 3 to 5, wherein
arbitrary foreign genes are integrated either as transcrip-
tion or as translation fusions.

7. Expression cassette according to claims 3 to 6, wherein the signal peptide of the SBP seed protein gene is used as a signal peptide.
- 5 8. Expression cassette according to claims 3 to 7, wherein the gene of the sucrose binding protein is used as the gene to be expressed.
9. Expression cassette according to claims 3 to 8, wherein it is also used for co- and multiple transformations.
- 10 10. Plasmids containing an expression cassette according to claims 3 to 8.
- 15 11. Plasmid pSBPROCS according to claim 10, comprising a DNA sequence about 5.3 kb in size, in which a SalI promoter fragment of the regulatory starter area about 1.9 kb in size including the signal peptide and 5 triplets of the SBP-homologous gene of *Vicia faba*, restriction sites for cloning of foreign genes and the transcription terminator of the octopine synthase gene are contained.
- 20 12. Plasmid pPTVSBPRGUS according to claim 10, a DNA sequence about 14.9 kb in size, in which a phosphinothricin resistance gene about 1 kb in size, a SalI/NcoI promoter fragment of the regulatory starter area of the SBP-like gene of *Vicia faba* about 1.8 kb in size, the coding region of the β -glucuronidase about 2 kb in size and the transcription terminator of the octopine synthase gene are contained.
- 25 30 13. Method for the insertion of an expression cassette according to claims 3 to 9 with a DNA sequence for strong seed-

specific gene expression into a plant cell, comprising the following steps:

- a) isolation of clone VfSBP20, wherein the gene coding for the SBP seed protein occurring in the plant seed is selected from a cDNA Bank of cotyledons of *Vicia faba*,
- b) isolation of clone pSBPR15, wherein the DNA sequence contained therein comprises the regulatory starter region of the SBP seed protein gene of *Vicia faba* and a sequence from a related legume hybridising with the DNA sequence of the SBPR15,
- c) production of the plasmid pSBPOCS making use of the Sall fragment of plasmid pSBPR15 1.9 kb in size,
- d) integration of foreign genes into the pSBPOCS expression cassette,
- e) cloning of the expression cassette containing a DNA sequence for over-expression of foreign genes in plant seeds into binary vectors
- f) transfer of the expression cassette containing an foreign gene under the control of the promoter according to claims 1 or 2 into a plant cell.

14. Use of an expression cassette according to claims 3 to 9 for expression of homologous and heterologous genes in the seeds of transformed plants.
15. Use of an expression cassette according to claims 3 to 9 for expression of genes changing the storage capacity or the germination capability of seeds.
16. Use of the plasmids pBISBPR7, pBISBPR15, pSBPGUS, pPTVSBPRGUS and pSBPOCS or derivatives thereof for transformation of culture crops.

17. Use of the plasmids pBISBPR7, pBISBPR15, pSBPGUS, pPTVSBPRGUS and pSBPOCS or derivatives thereof for regulation of endogenous processes or for production of heterogeneous products in culture crops.
- 5 18. Use of an expression cassette according to claims 3 to 9, wherein the transformed plants expressing new gene products or such altered in the seeds are selected, genetically stable lines are bred and the gene products are extracted from
- 10 the seeds of the transgenic plants.
19. Plant cell containing a plasmid according to claims 10 to 12.
- 15 20. Plant cell produced according to the method of claim 13.
21. Plant or plant tissues regenerated from a plant cell according to claims 14 or 15.
- 20 22. Plant according to claim 14, wherein it is a culture crop.
23. Use of the DNA sequence of the SBP signal peptide in an expression cassette for expression of arbitrary genes in
- 25 plant seed.

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Figure 1a

ATCCRACTCTGATCTTTGAATCTCTCTGTCCAACTGTTCTGAAGGAGTTCTAAGACTTTTCAGAAAGCTTTGTAAACAT
GCTTTGTAGACTTTCTTTGAATTAATCTCTGCAAACTCTGATTTGAACCTACGTGAAACTGCTCCAGAAAGTTCTTAACCAA
TTCCGCTCTGGGARGGCCCAAAATTTATTTAGTACTCTCAGTTTCATGGACGTGTCTTCAAGAGATTTTAACTTGAATCC
CATCATTTTAAAGAGAAAGTTCTGTTCGCCANTGTCTTAGATCTCATTTGAATCTACAACCTTGTGTCTCAGRAGTTCTTCC
AGAACTCAACTTGCATCATGGTGAATCTGGCCAGAACTTCTGAACCTTGTCAATTTCTTAAACAACTTTCATATTTCTAATCTA
AGTGTTAAGAATTTTGACTTTTCCAAAGCAACTTGTGACTTTCTTAAACAACTTTCATATTTCTAATCTAATCTAATCTA
CTTGATGAATGTGATTTCTTGAAATTTGATGTTGATGCAAAAGTTCGAATTTTGAATTTTGAATTTGAGTGTGGAAATTTGAGCAATTTT
GCTCTGTGTGCCAATTTCCAAACCTTAATTTGAATGATTCAGTGTCTGCARACTTGTATGTCTCATGGAAAGTCTTATGAGAAATTC
TTGAAGACTGAGAGGAAATTTTGTAGTACACACAAAGAACTTGTATGCTAGTCTGGACCTAGACACATTAACATAA
AACACCACTTCATTCGAAGAGTGTGTAAGAGGAAATTTTAAACAACTTAGAAGTAAATGGGAGTTAAAGAGCAACATTAAGG
ACTTTTACTAAATACACAAAGAGGAGATTTTAAACAACTTAGAAGTAAATGGGAGTTAAAGAGCAACATTAAGG
GGAGTGTAAATTAATGTGTGTGTAACCAACCACTTACCTTTAGTAAGTAAATGAGAAATTTGTAACTCACCATTATAT
TATGTCTCTTCTATGTTACTTTTCTTCAAGCCTATATAAAGCTTTGTAAGTAAATGAGAAATTTGTAACTCACCATTATAT
TATGTCTCTTCTATGTTACTTTTCTTCAAGCCTATATAAAGCTTTGTAAGTAAATGAGAAATTTGTAACTCACCATTATAT
ANTGAATTCATAGAAATTTATGGTATTTCAAGTCCAAATCCATCAATAGAAATTTAGTACAAACGTAACCTCAAAAT
ATTCTCTTATTTTAAATTTTACAACAATATAAATATTTCTCTTATTTTAAATTTTACAAATATAATTTATATCACCCTGT
CACCTTTAGAAATACCACCAACATATAATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTT
ATTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTTTATTT
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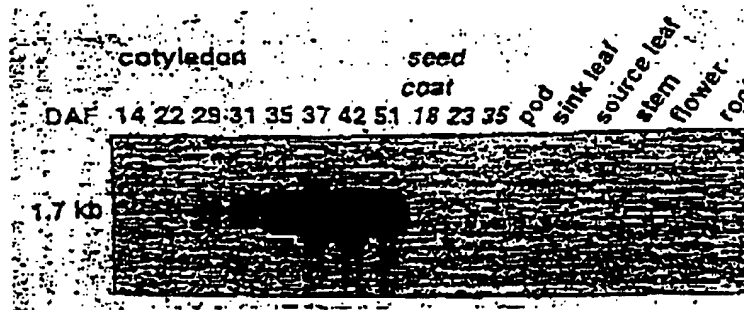
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Figure 2

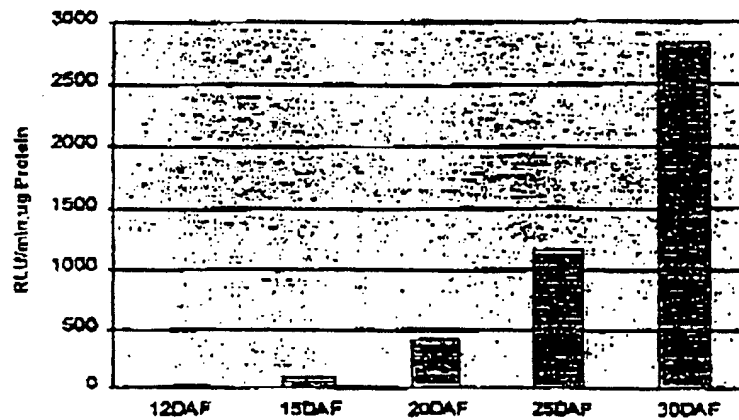


2a) Northern Blot of Vicia Faba Against VFSBP20 probe



2b) Cross-section of Ripe Transgenic (SBPRGUS) Tobacco Seed

β -glucuronidase Content in Transgenic pSBPRGUS Tobacco Line (n=15)



2c)

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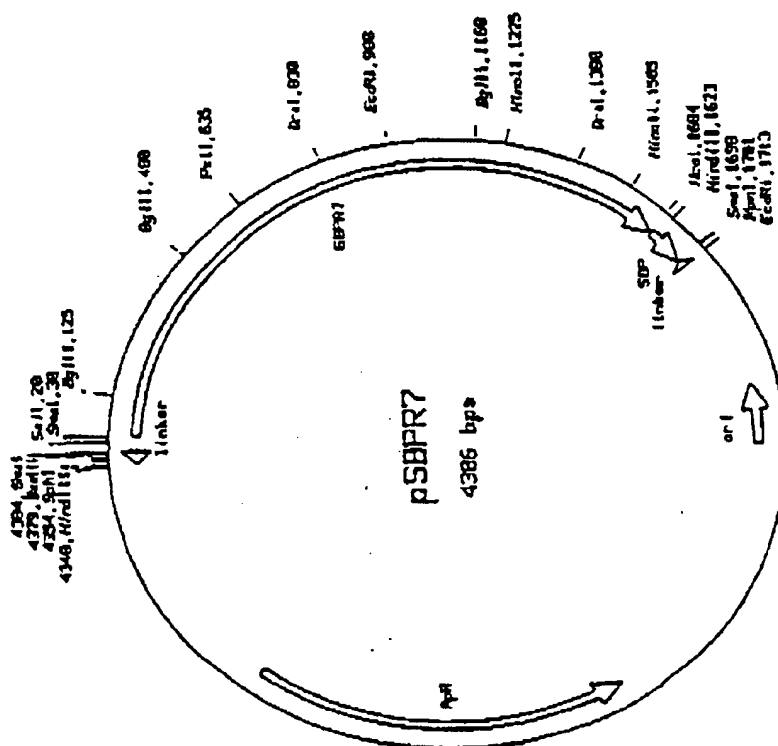
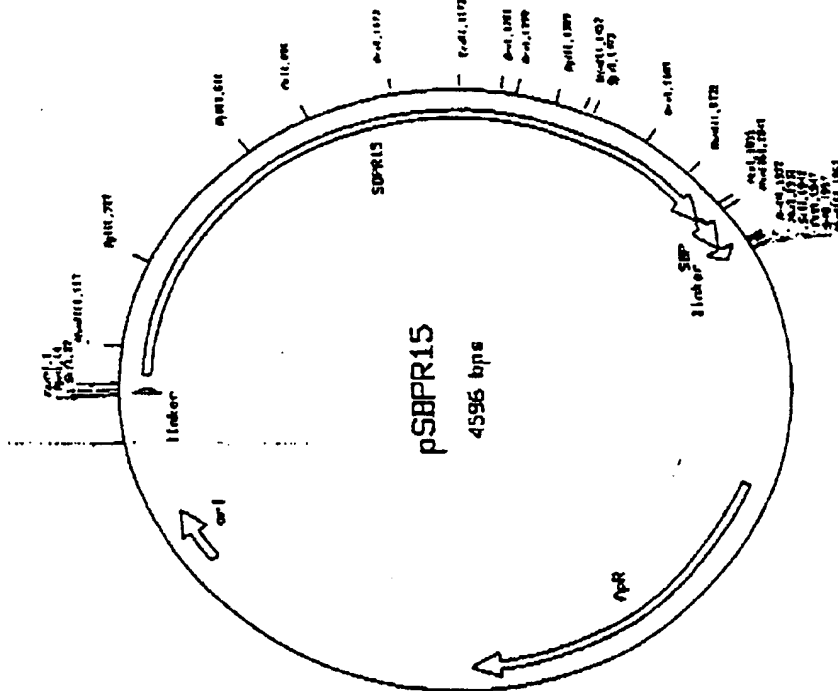


Figure 3

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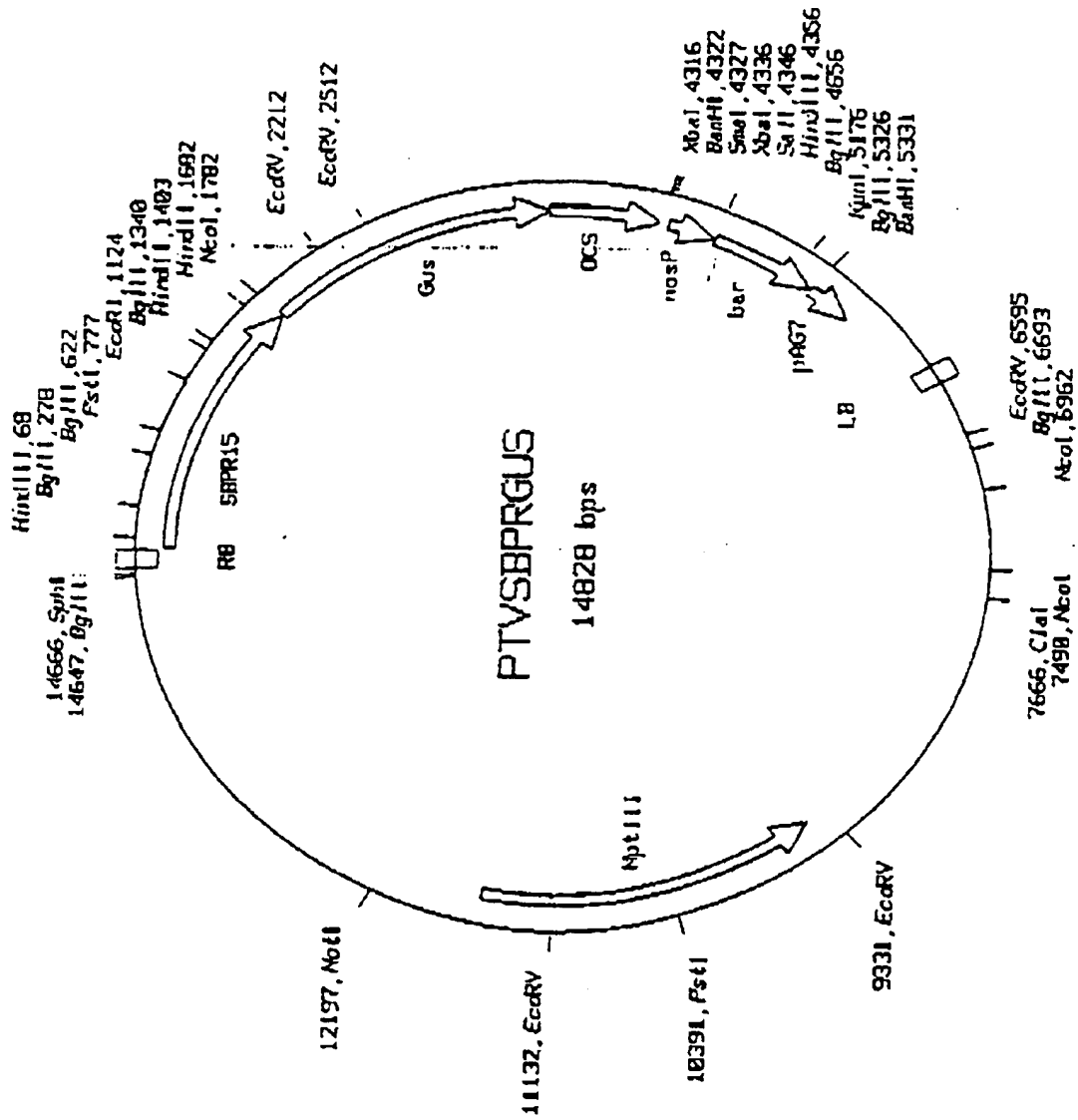


Figure 4

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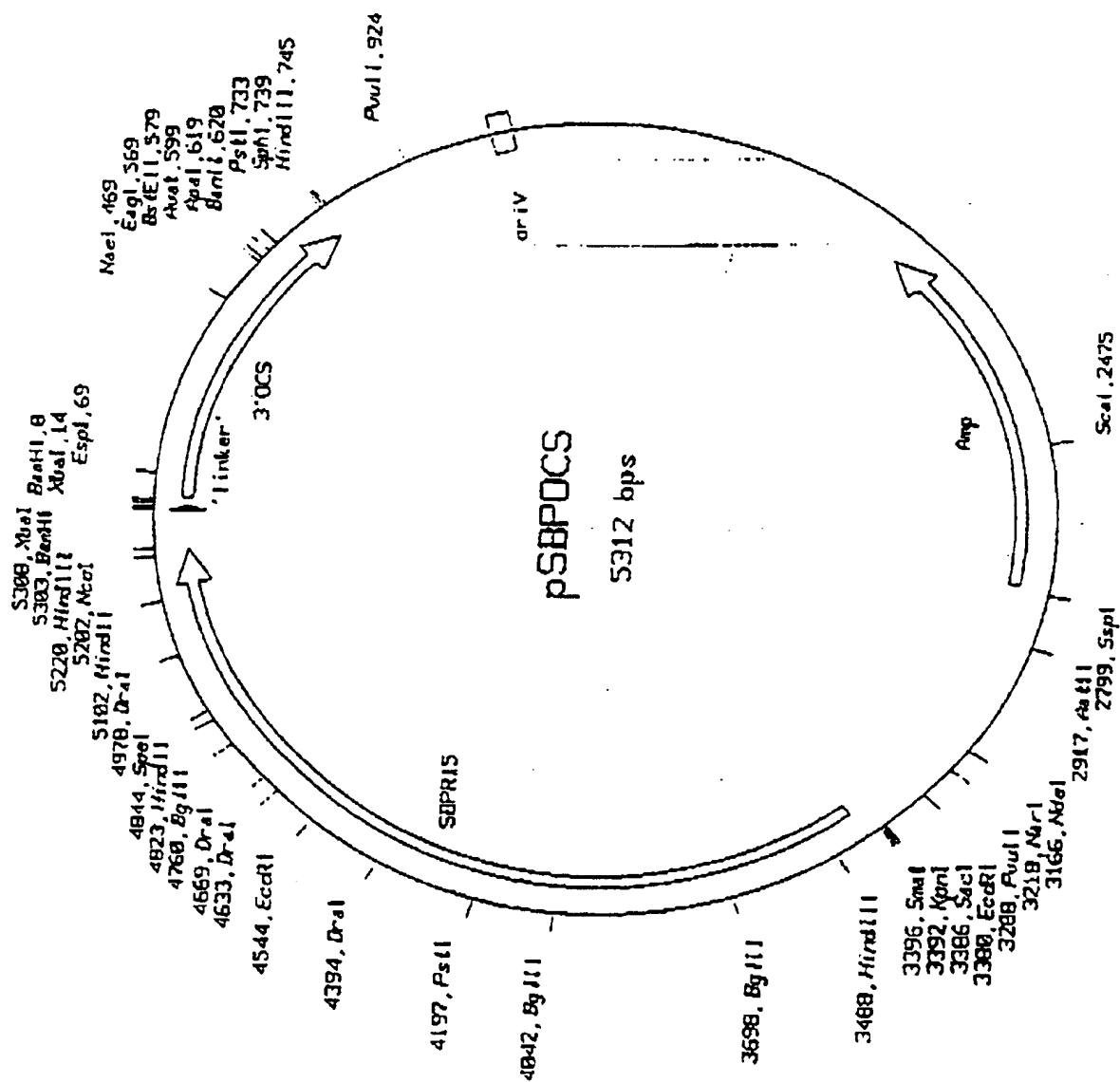


Figure 5

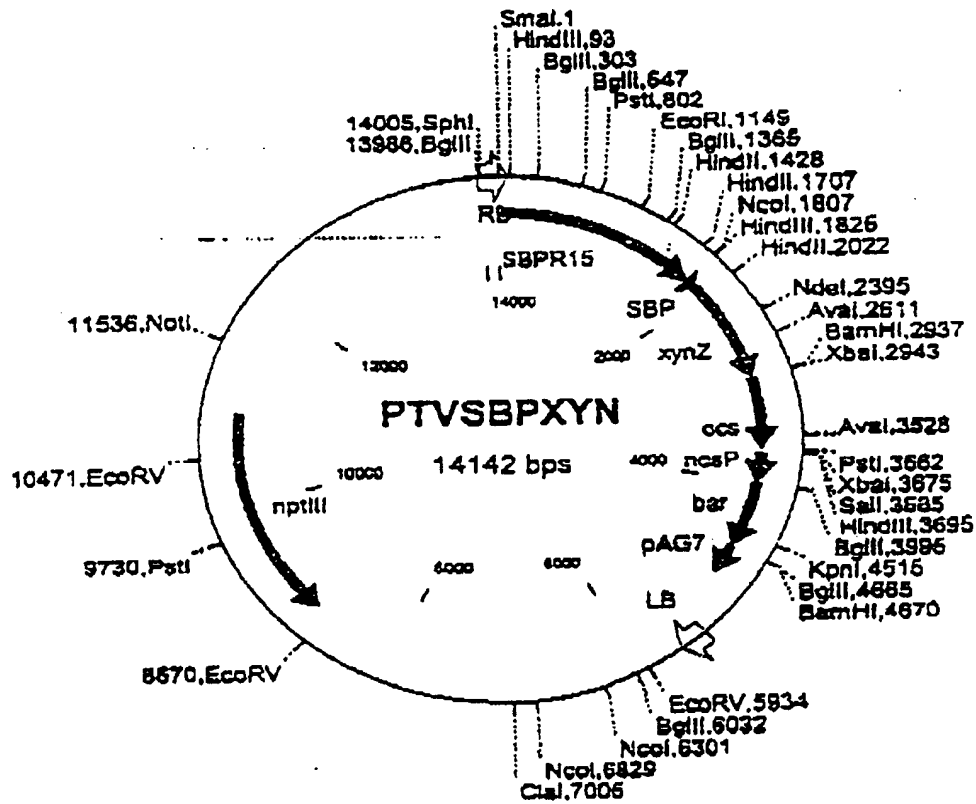


Figure 6

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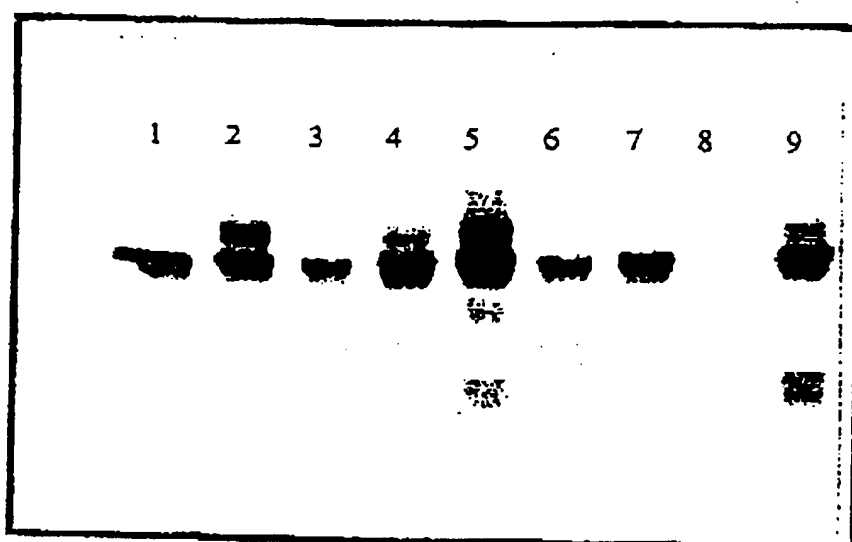


Figure 7: Western Blot of Protein Extract from Ripe Seed with Xylanase Z Directed Antibodies

Lanes 1-7 Independent with Plasmid PTVSBPXN Transformed N. Tabacum Line

Lane 8 Wild-type

Lane 9 Positive Control

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